Chapter 19. "Completing the knot"

Stress on enzyme-substrate complexes produced by matrix contraction and domain closure is relieved in catalysis by primary bond rearrangements converting substrates to products. When such stress is not necessary for catalysis or other physiological function, there is no reason to conserve the free energy and the true degree of affinity for a ligand is measured by the total free-energy change. The net free energy released is generally derived from matrix contraction so it is determined by the degree to which the ligand minimizes the entropy loss in that process. This is another critical trick found in evolution and one entirely depended on large size and precise tailoring of protein molecules. In this way matrix contraction provides a general mechanism for high affinity binding. and in view of the general association of "subtle-change" matrix contraction with C-2 symmetry of two dynamically balance domains, a mechanism that depends on control of domain closure in matrix contraction. The mechanical aspects of enzymic catalysis thus extend to strong and highly specific ligand binding related to steps of catalysis but identical only with the non-productive binding of strong specific inhibitors. The mechanism is less complicated than enzymic catalysis.In both selectivity depends on B values and their cooperative changes throughout large parts of proteins. Nature has made much greater use of entropy than has man.

The mechanical mechanism as applied to specific binding by proteins has been called "completing the knot" as used in protein folding but it is more often a manifestation of matrix contraction. It is exemplified by the pepsin-pepstatin combination already much discussed. When pepstatin or some equivalent ligand is not available, the protein contraction has low probability and occurs briefly and infrequently although as already mentioned the "entactic" phenomenon demonstrates that domain closure can occur spontaneously. Pepstatin eliminates

most of the pepsin free volume, almost all from matrices since knot free volumes are low. High affinity for this inhibitor must be attributed to matrix contraction rather than contact interactions. In this example and in general matrix contraction produces a hardness approaching that of the knots. Cartesian changes lie between 0.1 to 0.2Å, characteristic length change in primary-bond processes, and 0.5Å, the upper limit for the important changes in H-bonds and dispersion interactions. It is the B factors that provide estimates of the conformational contributions, information richly stored in the PDB but not yet much used. The errors in the values of positional coordinates hide the sophistication of matrix construction and explain why reliance on those coordinates and on analysis of structure in terms of secondary structures has been so unprofitable. On the positive side the small conformational changes in coordinates in function are probably not much different in dissolved states as in crystals so long as protein activity coefficients are not much altered.

Matrix or knot contraction can be found in one molecular form or another in many protein reactions from the tight binding of small ligands to protein-protein associations such as the "leucine zipper". There does not appear to be any alternate way to produce very high affinity for small or even large ligands without primary-bond formation. Similarly it is difficult to imagine another method for storage and manipulation of free energy. Ligand binding and matrix contraction are two sides of the same device. Ligands serve as triggers, locks and beneficiaries of matrix contraction. Ligand-independent protein-protein association can play the same roles. Hemoglobin illustrates not only ligand control but also redistribution of free energy among it subunits by rematching their tertiary structures. That is the classic example of "allosteric" interaction but any distortion of the conformation of one by another will change the free-energy distribution..

Primary-bond rearrangements can also produce strong and specific binding. It is interesting when it must depend on exchange of primary-bond free energy and conformational free energy, the "free-energy complementary" mechanism first suggested by Lumry and Biltonen. It is probably quite common but examples are few. The best of the latter is the conservation of potential energy in the combination of chymotrypsin with a sultone having a highly strained five-membered ring. In this elegantly conceived experiment Bolen and coworkers found an equilibrium binding constant near unity for formation of the acyl derivative with opening of the ring. Since the product was the original closed-ring substrate the 23 kcal/M of ring-strain potential energy was first retained in the acyl protein and then returned to substrate in reformation of the closed-ring substrate. Although the sultone is not a normal substrate, these results provide a rare lower estimate of the amount of free energy that can be stored in matrices. Presumable the free energy is stored by offsetting the normal expanding the matrices so as to replace matrix potential-energy by that released in primary-bond formation between sultone and protein. This important study has direct bearing on the deceptive nature of the binding of "transition-state analogs" The tight binding of such inhibitors is attributed to their congruency to the transition state on the assumption that adjustment of protein conformation is passive. No work has to be done on the inhibitor to improve its congruency in contrast to the stabilizing effect on the transition state with real substrates. The explanation is similar but reversed for mechanisms depending on destabilization of a pre-transition state. Any substrate analog already resembling the destabilized pre-transition state of its substrate cannot resist domain contraction so there is no increase in its potential energy and the free energy released by contraction is utilized in the binding process rather than in catalysis